Effect of Fasting and Refeeding on the Activities of Monoamine Oxidase and Antioxidant Enzymes in Rat Hypothalamus and Brown Adipose Tissue

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Abstract. Fasting for 48 h and the same period of recovery induced by 48 h refeeding increased rat hypothalamic monoamine oxidase (MAO) activity. However, in the interscapular brown adipose tissue (IBAT), only refeeding induced a significant elevation of the enzyme activity. As far as hypothalamic antioxidative enzymes are concerned, the copper zinc superoxide dismutase (CuZnSOD) activity was decreased in refed rats only. However, in the IBAT both food deprivation and refeeding induced a significant decrease in catalase (CAT) activity. Under the influence of fasting the adrenal glands were strongly activated as judged by the increased dopamine-beta-hydroxylase (DBH) activity and decreased cholesterol concentration. Refeeding brought both parameters to control levels indicating full recovery of these glands. As expected, fasting for 48 h induced a significant decrease in serum glucose but an increase in FFA concentrations. Thus, it can be concluded that both fasting and refeeding resulted in increased activation of hypothalamic MAO, whereas CuZnSOD activity was decreased only by refeeding. However, in the IBAT only refeeding increased MAO activity whereas both fasting and refeeding decreased that of CAT. In conclusion, it may be assumed that food deprivation for 48 h and the same duration of refeeding influenced MAO and antioxidative enzymes activities in the rat hypothalamus and IBAT in a tissue specific manner.

Key words: Fasting — Hypothalamus — IBAT — MAO — Antioxidants

Introduction

The tight association between feeding, metabolism, activity along the hypothalamic-pituitary-adrenal axis (HPA) and the sympatho-medullar system has been known for many years. A number of authors agree that there is a glucocorticoid response to food intake, both under basal and stress conditions, in most species studied (Krieger et al. 1971; Dallman 1984). However, studies into the influence of fasting

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on the HPA function and plasma glucocorticoid level have brought contradictory results. According to Dallman et al. (1993), high plasma glucocorticoid levels along with HPA axis inhibition are responses to acute stress following food restriction or deprivation in rats. On the contrary, other authors claim that fasting stimulates the activity of the HPA axis and reduces cortisol response to stress in humans (Adamson et al. 1989; Vance and Thorner 1989), sheep (Wronska et al. 1990) and rats (Honma et al. 1984). At the same time, it was shown that fasting elicits dissociation in the activity of the sympathetic nervous system (SNS) and the adrenal medulla. It decreases noradrenaline turnover in the brown fat and its urinary excretion, but increases adrenaline excretion (Davidovic et al. 1988; Davidovic 1992). Since brown adipose tissue plays a central role in the regulation of energy balance in small rodents (Rothwell and Stock 1979), the production of free radicals and consequently the activity of free radical scavenging enzymes in response to the altered oxidative metabolism might be changed. The main enzyme which dismutases superoxide anion radicals into H_2O_2 plus O_2 is superoxide dismutase (SOD). On the other hand, catalase (CAT) is also an antioxidant H_2O_2 scavenging enzyme, generated either during superoxide anion radical dismutation or by MAO catecholamine deamination (Cohen 1985, 1986).

For the reasons mentioned above, and the fact that brain catecholamines have an important role in the mediation of the glucocorticoid influence on HPA function, we examined the activity of monoamine oxidase (MAO), the main enzyme involved in catecholamine degradation, and that of antioxidative enzymes CuZnSOD, MnSOD and CAT in the hypothalamus and interscapular brown adipose tissue (IBAT) of rats exposed to fasting (48 h) and refeeding (48 h).

The functional changes in the adrenals were evaluated in terms of: 1. the activity of dopamine-beta-hydroxylase (DBH) in the adrenal glands, as the catecholamine synthesizing enzyme; 2. cholesterol (CHOL) concentration, as the precursor of glucocorticoid synthesis in the adrenal cortex; 3. the serum corticosterone (CORT) concentration, as the measure of hormone secretion. Other metabolic parameters, such as serum glucose (GLU) and free fatty acids (FFA) were also assessed.

Materials and Methods

Male rats (*Rattus norvegicus*) of Wistar strain, weighing 200 ± 20 g, 3 months of age were used for the experiments. The animals were acclimated to 22 ± 1 ℃, kept under intermittent 12 h periods of lights and dark, and given commercial rat food and water *ad libitum*.

The rats were divided into two groups, one group serving as controls ($n = 6$), kept during the whole experiment under the conditions mentioned above, and the other one was completely deprived of food for 48 h ($n = 12$), starting from 11 a.m. Half of the animals from the latter group were decapitated immediately after the completion of the 48 h fasting period, and the other half was kept under the condition of free access to food during subsequent 48 h and then sacrificed.

All animals were killed by decapitation at 11 a.m. with a guillotine (Harvard-Apparatus, U.S.A.), their heads were immersed into ice-cold bath, the brains were removed and kept frozen (−70 °C) for 1 month max. Before the analysis, the brains were melted and the hypothalami dissected. In this brain region, MAO activity and activities of the antioxidative enzymes CuZnSOD, MnSOD and CAT were measured. The activities of the same enzymes were also measured in IBAT.

MAO activity was determined according to the method of Wurtman and Axelrod (1963). The method consists of incubating the tissue homogenates at 37 °C with ^{14}C-tryptamine bisuccinate as the substrate, and measuring the radioactivity of ^{14}C-indol-acetic acid formed. Samples were counted in a liquid scintillation solution using an LKB scintillation counter. The radioactive material was purchased from New England Nuclear (NEN, UK). The obtained values were expressed as pmol of ^{14}C-indol-acetic acid per mg of tissue per min of incubation.

The brain tissue used for the determination of antioxidative enzyme activities was homogenized using 0.25mol/l sucrose; 0.05 mol/l TRIS and 0.1 mmol/l EDTA, adjusted with HCl to pH 7.4. The homogenates were sonicated 3 times at 100W for 20 s each at interval 10 s in a Bronson model B-12 sonicator, to release MnSOD, and then centrifuged at 37,000 rpm for 90 min in an ultracentrifuge (Ti50 rotor) for the determination of CuZnSOD and MnSOD activities by the adrenaline method (Misra and Fridovich 1972). This method is based on the measurement of adrenaline autooxidation inhibition rate by SOD contained in the examined samples in 50 mmol/l sodium carbonate buffer, pH 10.2, within the linear range of the autooxidative curve. One SOD unit was defined as the amount of the enzyme inhibiting adrenaline oxidation by 50% under the fixed reaction conditions of the assay. Total specific SOD activity and that of MnSOD activity, after inhibition with 4 mmol/l KCN, were measured, and then CuZnSOD activity was calculated. Catalase activity was measured by the method of Beutler (1982). This method is based on H_2O_2 degradation rate by the action of CAT contained in the examined samples followed spectrophotometrically at 230 nm in 5 mmol/l EDTA, Tris-HCl solution, pH 8.0.

The same methods were used to determine the activities of MAO, CuZnSOD, MnSOD and CAT in IBAT.

One adrenal gland was used for the determination of dopamine-beta-hydroxylase (DBH) activity, and another one for the measurement of cholesterol (CHOL) concentration. DBH activity was determined immediately after decapitation by the method of Kato et al. (1974, 1978), which consists of translating tyramine, under DBH effect, into octopamine which is oxidized in the presence of $NaIO_4$ into p-hydroxybenzaldehyde and formaldehyde. The concentration of the formed p-hydroxybenzaldehyde is measured spectrophotometrically and the enzyme activity is expressed in nmol/g tissue/min incubation. CHOL concentration was determined immediately after decapitation, according to the method of Zlatkis et al. (1953). The method consists of cholesterol reaction with ferric salts in concentrated sulfuric acid. The intensity of the formed violet color (560 nm) shows positive correlation with the cholesterol concentrations. The values are expressed as μg CHOL/mg tissue.

Blood glucose concentration was measured with an Exac-Tech glucose analyzer (MediSense Inc., Cambridge, MA, U.S.A.) using Dextrostix reagent strips. Serum FFA concentration was determined by the colorimetric method of Ducombe (1964). Serum corticosterone concentration was determined by a RIA kit (ICN Biomedicals, Costa Mesa, CA).

Statistics: One way ANOVA test was used to evaluate the experimental results. The values are presented as means ± S.E. of six animals, with $p < 0.05$ as the level of significance.

Results

Fasting of rats for 48 h induced a significant increase in DBH activity ($p < 0.01$) in the adrenal glands, suggesting the activation of the medulla and an enhanced catecholamine synthesis. After food deprivation, CHOL concentrations in the adrenals were significantly decreased ($p < 0.05$) as a result of intense glucocorticoid synthesis (Fig. 1). This increased synthesis was followed by insignificantly elevated corticosterone secretion as compared to controls (Fig. 2). The functions of the adrenal cortex and the medulla returned to the control levels after refeeding of the animals was started and continued for 48 h (Fig. 1).

A significant increase in MAO activity was observed in the hypothalamus ($p < 0.05$) after the 48 h fast in respect to controls. Interestingly, refeeding over 48 h did not bring MAO activity to the control level, being still significantly increased ($p < 0.05$) (Fig. 3). In the same brain region the 48 h fast did not induce significant

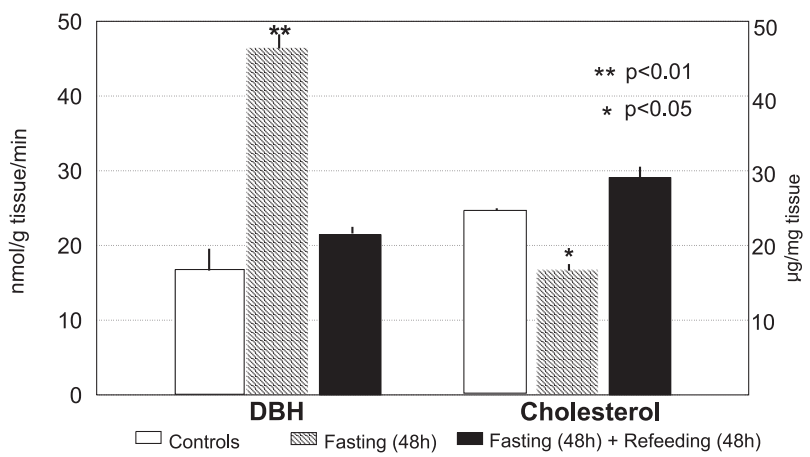

Figure 1. Adrenal DBH activities (nmol/g tissue/min) and cholesterol concentrations (μg/mg tissue) in control, fasted (48 h) and refed (48 h) rats. Data points represent means and S. E. of values obtained for 6 animals. *$p < 0.05$; **$p < 0.01$.

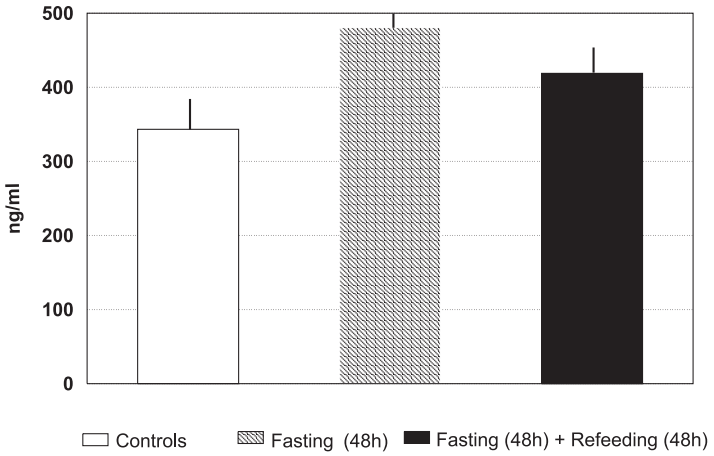

Figure 2. Serum corticosterone concentrations (ng/ml) in control, fasted (48 h) and refed (48 h) rats. Data points represent means and S. E. of values obtained for 6 animals.

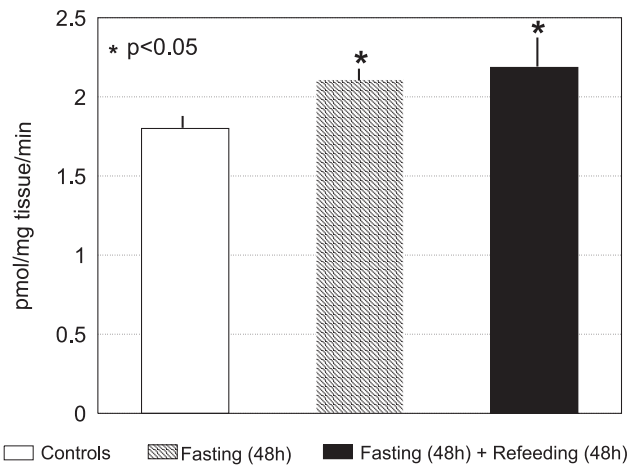

Figure 3. Hypothalamic MAO (pmol/mg tissue/min) activities in control, fasted (48 h) and refed (48 h) rats. Data points represent means and S.E. of values obtained for 6 animals. $^*p < 0.05$.

changes in CuZnSOD, MnSOD and CAT activities, whereas refeeding of fasted animals decreased CuZnSOD activity ($p < 0.05$) (Fig. 4).

As evident from Fig. 5, MAO activity in IBAT was not changed under the influence of fasting, but was significantly elevated after the refeeding recovery period ($p < 0.01$). In this fat tissue CAT activity was significantly diminished under the

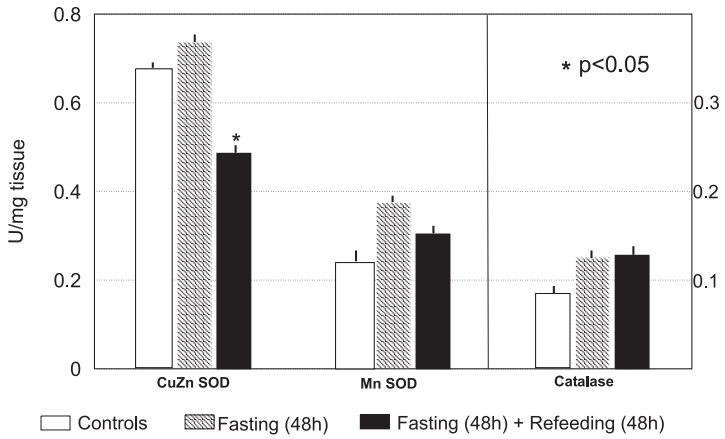

Figure 4. Hypothalamic CuZnSOD, MnSOD and catalase activities (U/mg tissue) in control, fasted (48 h) and refed (48 h) rats. Data points represent means and S.E. of values obtained for 6 animals. $^{*}p < 0.05$.

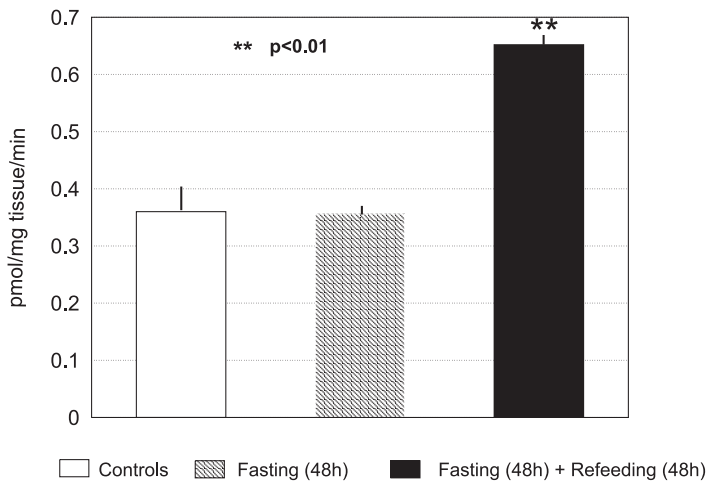

Figure 5. IBAT MAO activities (pmol/mg tissue/min) in control, fasted (48 h) and refed (48 h) rats. Data points represent means and S.E. of values obtained for 6 animals. $^{**}p < 0.01$.

influence of food deprivation ($p < 0.01$), being still below the control level after food intake over 48 h ($p < 0.05$), but much above the values obtained in fasted animals ($p < 0.05$). The IBAT CuZnSOD and MnSOD activities remained unchanged both after food deprivation and refeeding (Fig. 6).

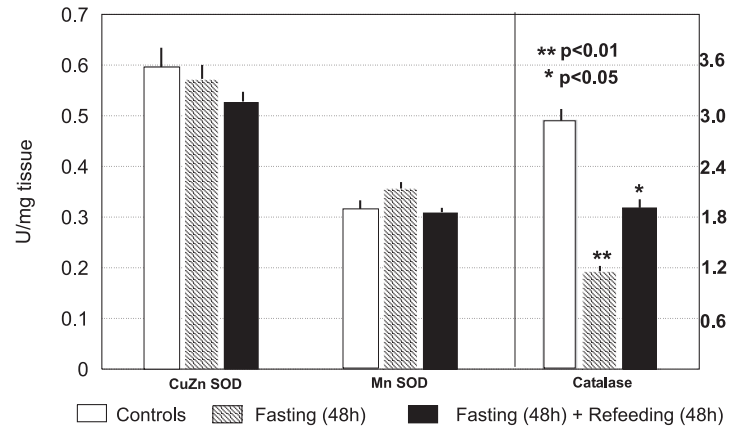

Figure 6. IBAT CuZnSOD, MnSOD and catalase activities (U/mg tissue) in control, fasted (48 h) and refed (48 h) rats. Data points represent means and S.E. of values obtained for 6 animals. $*p < 0.05$; $**p < 0.01$.

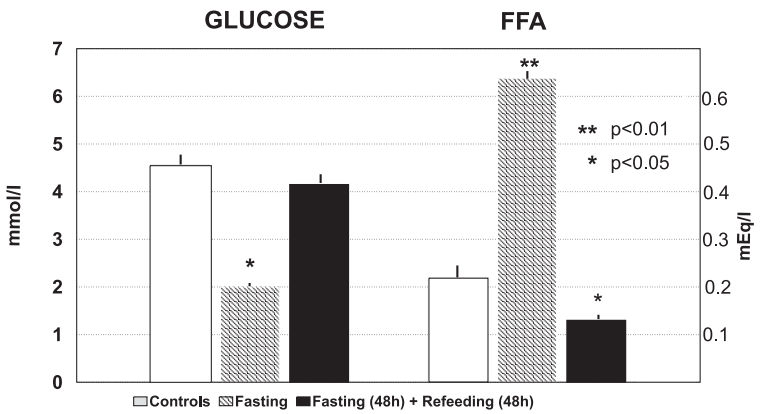

Figure 7. Serum glucose (mmol/l) and FFA (mEq/l) concentrations in control, fasted (48 h) and refed (48 h) rats. Data points represent means and S.E. of values obtained for 6 animals. $*p < 0.05$; $**p < 0.01$.

As expected, fasting for 48 h induced a significant decrease in serum GLU ($p < 0.05$) and increase in FFA ($p < 0.01$) concentrations. After 48 h refeeding, GLU reached control levels but FFA dropped even below the levels observed in fasting rats ($p < 0.05$) (Fig. 7).

Discussion

The results of our studies show that fasting (48 h) activates the adrenals (cortex and medulla) which are the functional part of the HPA axis. This response corresponds with the results of Honma et al. (1984) and Vance and Thorner (1989) who reported that food deprivation stimulates the activity of the HPA axis. The effect of refeeding on previously fasted animals completely restored CHOL and DBH levels in the adrenal glands. Surprisingly, the corresponding elevation of serum corticosterone concentrations in response to enhanced adrenal function, observed 48 h after the beginning of food deprivation, was not significant. This was probably due to time dependent variations in corticosterone secretion following food deprivation or/and to its half-life. Dallman et al. (1994) recorded two major episodes of ACTH and corticosterone secretion during a 14 h overnight fasting as compared to control rats fed *ad lib*, one being 90 min after the onset of the dark cycle and the other one about 3 h prior to switching lights on. Akana et al. (1994) claim that the marked response of the HPA axis during a short period of fasting served as a default pathway for neural activity that usually subserves feeding behavior.

Glucocorticoids exert their effect on catecholamines in the brain (Petrovic et al. 1993) either by regulating their synthesis (Cvijic et al. 1988) or degradation (Cvijic et al. 1993). It is well documented that exposure to various physiological stimuli that trigger the activation of both the HPA axis and the sympathoadrenomedullary system results also in an overall enhancement of the activity of central noradrenaline-containing neurons (Lachuer et al. 1991). The main enzyme which is involved in the oxidative deamination of biogenic amines secreted from the monoaminergic nerve terminals is MAO (Gordis and Neff 1971). The enzyme operates to maintain neurotransmitter concentrations at a basal level. In our experiment, food deprivation for 48 h induced a significant increase in hypothalamic MAO activity. This elevation of MAO might be the consequence of intense catecholamine secretion in these brain regions, as a response to CORT secretion from the adrenal cortex induced by fasting. It was shown that 24 h after the application of this hormone, hypothalamic MAO-A activity was elevated by more than 100%, which not only suggests higher monoamine concentrations and deamination of the substrate but also a marked change in its physiological role (Cvijic et al. 1993). According to Waldmeir (1987), changes in MAO activity, only if higher than 80%, can imply a changed physiological role of the adequate substrate. It is noteworthy that in the present experiment MAO activity remained at a much higher level than in controls even after 48 h refeeding. It is possible that some indirectly acting food amines (e.g. tyramine) induced prolonged MAO activation, given that in patients treated with MAO inhibitors this amine sympathomimetic action, known as "cheese effect", is potentiated (Youdim and Finberg 1991).

Bearing in mind that in our experiments the hypothalamic MAO activity was elevated under the influence of fasting we also expected CAT to be elevated. However, after food deprivation for 48 h, there were no significant changes in the activity of either this antioxidant enzyme or of the other two enzymes (CuZnSOD

and MnSOD). Another H_2O_2 removing enzyme, glutathione peroxidase, may have been involved, since catalase activity in the brain is markedly low (Halliwell and Gutteridge 1985). A significant decrement in the hypothalamic CuZnSOD activity was observed after the 48 h refeeding period. As mentioned above, under the same experimental conditions, MAO activity was still very high producing correspondingly high H_2O_2 concentrations, H_2O_2 being known to inhibit CuZnSOD activity (Halliwell and Gutteridge 1989).

Fasting has been reported to induce depression of the sympathetic nervous system, as judged by the marked decrease in noradrenaline turnover in many adrenergic innervated tissues such as IBAT (Young et al. 1982). This is in agreement with our findings on the unchanged MAO activity in IBAT: NA is known to be the preferred substrate for this enzyme and MAO is involved in its degradation in IBAT. Davidovic et al. (1988) and Davidovic (1992) also showed that fasting for 48 h reduced noradrenaline urinary excretion. However, under the same experimental conditions, marked elevation of urinary adrenaline excretion and adrenal noradrenaline content were observed (Davidovic et al. 1988). This is in accordance with the increased adrenal DBH activity observed in our present experiments, induced by 48 h fasting. Thus, the increase in serum FFA in food deprived animals does not seem to be mediated by the sympathetic nervous system but by an increased adrenaline secretion from the medulla. Hypoinsulinemia, induced by low glucose levels, might contribute to these processes given that it stimulates IBAT to become more sensitive to the lipolytic effects of adrenaline during short-term fasting (Jensen et al. 1987; Wolfe et al. 1987). The addition of a small amount of glucose was shown to reduce the increased serum FFA concentration in fasted animals (Paschoalini and Migliorini 1990). Under fasting conditions IBAT "rests" conserving energy. This has been evidenced by the results of Gong et al. (1997) who showed that the contents of the uncoupling proteins (UCP_1 and UCP_3) are very low in IBAT of fasting animals. Both SODs' activities in IBAT were unaffected by fasting, whereas that of CAT was markedly decreased. This might be explained by the fact that when SNS activity is low, β-oxidation in IBAT peroxisomes may be depressed, and since CAT is the indicator of this process its activity is also low. However, as judged by the very high IBAT MAO activity in the refed rats, this tissue is strongly activated by SNS, inducing an increased CAT activity as compared to that in IBAT of fasting rats. This is in accordance with the findings of Davidovic (1992) and Davidovic et al. (1992) who found high noradrenaline urinary excretion and an increased IBAT noradrenaline turnover under similar experimental conditions. At the same time, the amounts of serum FFA rapidly decreased in refed animals, being even lower than in the controls indicating its increased utilization. Recently, Gong et al. (1997) showed that UCP_1 and UCP_3 contents increased when food was provided to previously fasted animals. This suggests that energy processes get activated in this tissue.

Thus, we can conclude that IBAT participates in the regulation of metabolic substrates in fasted rats, which is in agreement with the results of Davidovic et al. (1988) and Cooney and Newsholme (1982) who reported that IBAT is an important

site of glucose utilization and its conversion into lipid (Trayhurn 1981). Besides, both fasting and refeeding induced significant increases in hypothalamic MAO activity, but had no influence on the activities of the brain antioxidant enzymes studied. However, the increased MAO activity in the hypothalamus correlates with the increased HPA function in fasted animals only.

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